1445

GROWTH FACTOR LEVELS IN THE DIABETIC VITREOUS: CORRELATION WITH TYPE OF DIABETES AND NEOVASCULAR ACTIVITY

BOULTON M.,[1] GREGOR Z.,[2] MORIARTY P.,[1] KHALIQ A.[1], CHARTERIS D.[2] and McLEOD D.[1]
[1]Department of Ophthalmology, Manchester Royal Eye Hospital, Manchester (UK)
[2]Moorfields Eye Hospital, City Road, London (UK)

Purpose. To determine growth factor levels in individual vitrectomy samples from insulin-dependent and non-insulin dependent diabetics (IDDM & NIDDM) and to correlate such levels with a) the type of diabetes and b) neovascular activity.
Method. Over 70 vitreous gel samples were obtained from diabetic patients with proliferative retinopathy and non-diabetic ("control") patients undergoing vitrectomy. Levels of bFGF, IGF-I, EGF, TGF-β2 and insulin were determined by radioimmunoassay. IGF-I binding protein levels were assessed by laser densitometry of Western ligand blots.
Results. bFGF levels were significantly greater in vitreous samples from NIDDM patients than either IDDM or non-diabetic patients. When subdivided into eyes with active neovascular or inactive fibrotic membranes the highest levels of bFGF were found in NIDDM patients with active membranes. No significant difference in the levels of the other growth factors was observed between the NIDDM and non-diabetic group. In the IDDM group, both IGF-I and a 34kDa IGF-BP were elevated compared to the other groups while TGF-β2 levels were greatly reduced. The increase in IGF-I and IGF-BPs, and the decrease in TGF-β2, were greatest in patients with active neovascularisation. bFGF and EGF levels were did not differ between the IDDM and non-diabetic groups.
Conclusion. In proliferative diabetic retinopathy there is a correlation between intravitreal growth factors and both disease type (whether IDDM or NIDDM) and disease state (whether active or fibrotic).

1446

TITLE: CHANGES OF THE PROTEIN REDOX STATUS IN THE VITREOUS OF DIABETIC PATIENTS.

AUTHORS: CARDIA L.[1] MICELLI FERRARI T.[1] BOSCIA F.[1] CARDIA G.[1] GRATTAGLIANO I.[2] VENDEMIALE G.[2] ALTOMARE E.[2]
1: ISTITUTO DI CLINICA OCULISTICA - UNIVERSITA' DI BARI - ITALY
2: ISTITUTO DI CLINICA MEDICA I - UNIVERSITA' DI BARI - ITALY

BACKGROUND: Structural changes of proteins are considered among the basic mechanism leading to diabetic complications. However, little is known about the redox status of the vitreous proteins in diabetic subjects. Therefore, the aim of this study was to investigate the content of sulfhydryl proteins (P-SH), protein mixed disulfides (PB-SH) and carbonyl proteins (DNPH) in the vitreous of diabetic and non diabetic subjects.
METHODS: 27 subjects were included in the study (A=7 healthy controls, B=10 type-2 diabetic subjects affected by proliferative retinopathy; C=10 type-2 diabetic subjects affected by non-proliferative retinopathy). After suction the vitreous was centrifuged and the supernatant analyzed for PB-SH and DNPH, indices of oxidatively demaged proteins, and P-SH, index of reduced status of the proteins.
RESULTS: A significant lower content of P-SH was found in the vitreous of diabetic subjects (9.7±2.4 vs 17±5.4 nmol/mg prot, p 0.001), especially when a retinal complication was present (7.1±2.1 nmol/mg prot, p 0.02 compared to C). Moreover an increased formation of PB-SH and DNPH was noticed in diabetic subjects compared to healthy controls (p 0.001). Yet, these parameters resulted to be particularly altered when diabetes was associated with retinal disease.
CONCLUSIONS: This study shows an alteration of the protein redox status in subjects affected by diabetes mellitus. Vitreous proteins were found to be oxidized to a larger extent in the presence of retinal disease.

1447

PLASMINOGEN ACTIVATOR AND INHIBITOR EXPRESSION IN HUMAN RETINAL VASCULAR ENDOTHELIAL CELLS IN VITRO

REDMILL B.S.[1,2] BOOTH N. A.[1] KNOTT R. M.[2] FORRESTER J. V.[2]
[1]Department of Molecular and Cell Biology, University of Aberdeen, Scotland
[2]Department of Ophthalmology, University of Aberdeen, Scotland

Purpose Localized, plasmin-mediated extracellular matrix proteolysis on the surface of vascular endothelial cells is an important feature of angiogenesis. We report on the expression and localization of plasminogen activators, their inhibitors and cell surface receptors by human retinal endothelial cells.

Methods Retinae were homogenised, incubated with collagenase, and cultured on fibronectin coated plastic. Plasminogen activators and their inhibitors were detected in endothelial cell culture supernatants by SDS-PAGE with zymography using fibrin-agarose plates. These proteins were quantified by specific ELISA. The proteins were detected on cell culture monolayers by immunocytochemistry using alkaline phosphatase and by immunofluorescence with scanning laser confocal microscopy and conventional fluorescence microscopy. Expression at the level of mRNA was studied by PCR using specific primers.

Results Human retinal endothelial cell monolayers stained positively for plasminogen activator inhibitor-1 (PAI-1), urokinase type plasminogen activator (u-PA), u-PA receptor, tissue type plasminogen activator (t-PA) and plasminogen activator inhibitor-2 (PAI-2). mRNA for u-PA and PAI-1 was detected. Cell culture supernatants contained in the range of 100ng/ml PAI-1, 10ng/ml u-PA and 1 ng/ml t-PA.

Conclusions These results suggest that plasmin generation could occur on the cell surface but would be limited in the fluid phase. Regulation of plasminogen activation by endothelial cells is critical in angiogenesis and this system offers the potential to characterise these mechanisms and their role in the development of retinal neovascularisation.